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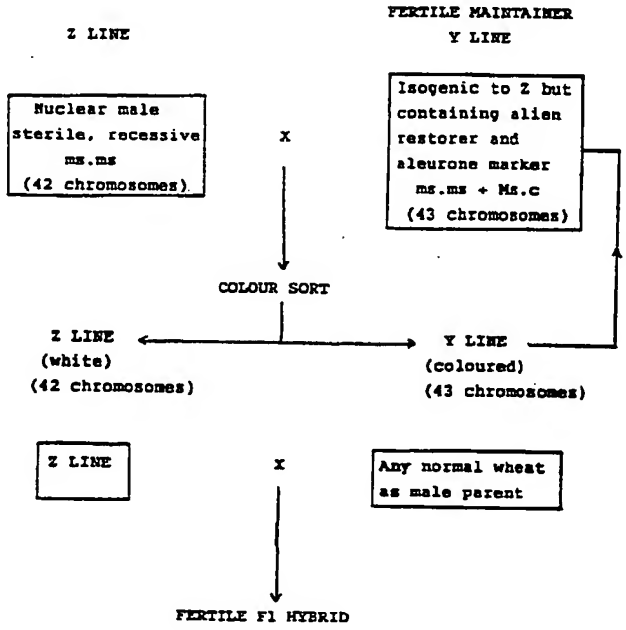
(54) Title: PRODUCTION OF HYBRID CEREAL CROPS




(57) Abstract

This invention relates to the production of hybrids of cereal crops, particularly small grained cereals such as wheat and barley. In addition, the invention concerns new genetically altered plants, for use with this method. The plant line used in the production of hybrids, has a chromosome bearing a dominant male fertility restorer gene and a colour marker gene which confers a characteristic colouration on the progeny seed. The male sterile parental plant line is maintained by physically separating the progeny seeds by colour sorting.

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## + DESIGNATIONS OF "SU"

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# PRODUCTION OF HYBRID CEREAL CROPS

## TECHNICAL FIELD

This invention relates to the production of hybrids of cereal crops, particularly, but not exclusively, small grained cereals such as wheat and barley. In addition, the invention concerns new genetically transformed plants, for use with this method.

## BACKGROUND ART

The traditional manner of producing hybrid plants involves manual emasculation of the female parent, so that self-pollination is not posible, which is planted proximate the fertile male parent. This procedure is only practical when it is possible to remove the pollen bearing structures from the female parent. In many species, however, the flowers are so insignificant in size that manual emasculation of the female parent of an intended cross is simply impracticable. This is particularly so with small-grained cereals such as wheat, barley, rice and grass species.

When physical removal of the anthers from the female parent is impracticable, sterility may be induced by treatment of the plant with a chemical hybridising agent (CHA) which inhibits synthesis of viable pollen. Compared with physical emasculation, CHA treatment is somehwat inefficient and a certain amount of self-pollination occurs which results in the harvested hybrid seed containing some seed of the female parent. The male parent is normally grown in clearly indentifiable rows or blocks and are simply not harvested; the male parents thus present no problem to hybrid purity. As a generalisation then, the seed hybrids which are produced using CHAs tend to be contaminated with seed of the female parent and separation of this rogue contaminant is virtually impossible.

A useful alternative procedure which is available to plant breeders utilises the phenomenon of cytoplasmic male sterility (CMS). This type of male sterility arises from

genetic material present in the cytoplasm of plant cells. It is rare for genetic information from the cytoplasm to be transferred via the pollen to the zygote during pollination, as the cytoplasm of the zygote arises almost exclusively from the female parent. When a plant carrying CMS is used as a
female parent in a cross, the progeny all possess the CMS trait. In hybrid production, CMS inbred lines are crossed with pollinators which possess a nuclear encoded "restorer" gene which inhibits expression of the male sterility characteristic encoded in the cytoplasm and, therefore, yields male fertile progeny. Therefore, the progeny still retain the male sterility genetic material in the cytoplasm; expression is suppressed by the dominant male fertility gene in the nucleus. An example of CMS system will be found in United States Patent Number 2,753,663 which describes the production of hybrid maize by this method.

An alternative to the CMS system has been proposed [Driscoll, G.J.; Crop Science 12, 516-517 (1972)] and is known as the XYZ system,. This system avoids the introduction of alien genetic or cytoplasmic material in the final product seed. The XYZ system is itself an extension of an even earlier proposal described by Ramage R.T.; Crop Science 177-178 (1965). The XYZ system is illustrated in Figure 1 of the accompanying drawings. The system employes

(1) a homozygous genically sterile female parent (the Z line);

(2) a Y line which is isogenic with Z line but possesses an additional unpaired chromosome carrying a male fertility restorer gene; and

(3) an X line which is identical with the Y line but has two doses of the restorer gene.

Initially the Z and X lines are crossed to produce the Y line, Then the Y line is crossed with the Z line giving progeny of which 80% are of the Z line genotype and 20% of the Y-type genotype. This mixed population is then used in a cross with any desirable male fertile line to produce the commercial F1 hybrids. The principal advantage of the XYZ

system is that the alien restorer chromosome is eliminated by physical separation and does not apper in the commercial hybrid.

In Driscoll's original proposal for hybrid wheat production by the XYZ system, the alien restorer chromosome which was selected was chromosome 5R from cereal rye, Secale cereal L.. Chromose 5R from some rye cultivars was demonstrated to produce normal male fertility when substituted for chromosome 4A in bread wheat, Triticum aestivum L.. Male sterility in the XYZ system is the product of simply inherited recessive mutation on the short arm of chromosome 4A (4As). The advantage of using chromosome 5R of a gene restorer chromosome was the presence on 5R of a gene producing a hairy peduncle (Hp) which allows identification of any plants carrying the alien restorer chromosome to be identified visually. However, as will be appreciated, this is only a suitable marker when working with small populations.

Driscoll subsequently proposed a modified XYZ system for wheat ["Modified XYZ System of Producing Hybrid Wheat" Crop Science 25, 1115-1116 (1985)]. In this system it was stipulated that the alien chromosome was to be an isochromosome. For the purpose of the present invention this modification is not of any particular relevance.

An object of the present invention is to obviate or mitigate the aforesaid disadvantages.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the scheme of the Driscoll XYZ system, as discussed previously.

Figure 2 shows an aspect of the system of the present invention using an addition plant line.

Figure 3 shows another aspect of the system of the present invention using a substitution line and a 3-way cross system.

Figure 4 shows another aspect of the system of the present invention using a substitution line and a single cross.

DISCLOSURE OF INVENTION

According to the present invention there is provided a method for the maintenance of a male sterile parental plant line for use in the production of hybrids, comprising crossing a homozygous male sterile plant, representing the female parent, with a male parent which is isogenic to the female but having a chromosome bearing a dominant male fertility gene and a marker gene which confers a characteristic colouration on the progeny seed, harvesting from that cross a population of progeny seed consisting of a mixture of the two parental lines, and physically separating the progeny seed on the basis of the colour marker.

The chromosome bearing the dominant male fertility gene, may be an additional gene, so that for example, in wheat the female has 43 chromosomes, or else may be a substitution, whereby the female has the usual number of chromosomes; for example, 42 for wheat.

Separation of the mixed population of progeny seed may therefore be effected using commercially available seed-sorting machinery which is capable of colour discrimination.

The present invention is particularly applicable to the production of hybrid wheat and barley. However, it will be appreciated that the method of the invention may be applied to any plant species for which the appropriate starting materials exist or may be created. Other such species include rice, maize and grass species.

It is preferred that the colour marker be the blue aleurone marker gene available on chromosome 4 of Agropyron elongatum (4Ag). The male parent used in this invention is suitably a translocation of 4Ag to the restorer arm of chromosome 4 of Triticum thaoudar (4th) and chromosome 4 of Tricicum monococcum (4m), both of which are diploid wheats or to the restorer arm of chromosome 4 of Triticum urartu.

Blue markers are known also to exist within barley germplasm, as do the necessary genetic male sterility genes. United States Patent Number 3,710,511 (Patterson) shows that a range of suitable stocks are known to be

available for other crop species and are described in the literature.

The present invention may be viewed as a modification of the XYZ system of Driscoll. Driscoll's proposal to use rye chromosome 5R effectively limited the size of the Z populations. This is because of the need for intense roguing to remove plants carrying the hairy peduncle: similar roguing would be necessary in the commercial production phase.

In comparison, the present invention utilises a distinctive colour marker such as the blue aleurone gene from Agropyron elongatum L. (4Ag). However, it has previously been shown that chromosome 4Ag substitutions were vigorous but male sterile. This indicates that 4Ag lacks the gene for male fertility restoration, necessary to restore the Z line in Driscoll's XYZ system, which has been confirmed in recent tests.

However, a translocation between 4Ag and the restorer arm of alien restorers Triticum monococcum (4m), Triticum thaoudar (4th) or Triticum urartu (4u) have been located and have been found to carry both the blue aleurone marker (BL) and a male fertility restorer (+MS). Crossing of the translocation into a male sterile line provides a suitable material for use in the present invention.

In addition, propagation of the addition plant lines can give rise to a substitution line, particularly after self-pollination for several generations. The substitution possibly occurs by centric fusion or Robertsonian translocation, for example, by the substitution of one arm from a Agropyron elongatum L. chromosome with one arm from T. monococcum or T. thaoudar.

The substitution plant line has some further advantages in comparison to the addition line. In the first system described above, the nuclear male sterile (NMS) system is based on an addition line (43 chromosomes, while bread wheat normally carries 42 chromosomes). However, an addition line may have some disadvantages particularly with seed production. When an addition line (in which the 43rd

chromosome carries the blue aleurone marker(s) and the male fertility restorer) self-pollinat s the progeny will be approximately 72% non-blue and male sterile. Of the remnant 27% will be a monosomic addition, ie, 43 chromosomes (one dose of blue), and 1% will be homozygous blue, ie, 44 chromosomes. Through colour sorting, the 28% blue progeny can be separated. In spite of this, the rate of increase in seed volumes is quite slow when using an addition line.

However, while this is still satisfactory, it can be improved upon, since calculations using a substitute line show a more favourable result. A substitution line consists of the normal 42 chromosome complement of bread wheat, for example, in which a wheat chromosome is replaced by a non-bread wheat chromosome. This means that the segregation ratio will approximate the Mendelian ration of 3:1, and so approximately 75% will be blue, and 25% non-blue.

MODES FOR CARRYING OUT THE INVENTION

The present invention will now be described, by way of illustration, with reference to the following Examples.

EXAMPLE 1.

(a) Production of Z and Y Lines

A nuclear male sterile line of variety PROBUS was backcrossed into a variety of cultivars to introduce the male sterile recessive genotype thereto. This provided a series of lines constituting the Z line for use in the breeding scheme of this invention. The cultivars were: HARRIER, SUNECA, VASCO, TORRES, SUNSTAR, BANKS, SKUA, AROONA, VULCAN AND TORDO.

The Y line required for this example was created as follows: Chinese Spring Wheat was crossed with Agropyron elongatum or another alien species such as Triticum thaoudar, Triticum monococcum or Triticum urartu and haploid plants selected from amongst the progeny. The haploids were treated with colchicine to induce chromosome doubling. By this method there were obtained the following addition lines:

Chromosome 4 - Triticum urartu in male sterile Chinese Spring Wheat;

Chromosome 4 - Triticum monococcum in male sterile Chinese Spring Wheat; and,

Chromosome 4 - Triticum thaoudar in male sterile Chinese Spring Wheat.

These doubled haploid plants were then self-pollinated or, alternatively, backcrossed to one of the Z line wheats described above and plants bearing single alien additions were selected.

These lines were then crossed with Agropyron containing a blue aleurone gene in order to create centric fusions which carry both the male fertility gene and the blue marker.

These then provide the Y lines which are made up of the Z lines plus the restorer/blue chromosomes.

(b) Production of Hybrids

The breeding scheme of the present invention is illustrated schematically in Figure 2 of the drawings herewith.

The nuclear male sterile recessive Z-line of wheat, having the genotype ms.ms was crossed with a fertile maintainer line (designated the Y line), isogenic with the sterile line (ms.ms) but containing one alien chromosome capable of restoring fertility and carrying a blue aleurone colour marker derived from Agropyron elongatum (genotype Ms.c).

What was obtained from that cross was a mixture of normal (white) and blue coloured seed which had resulted from imperfect transmission of the alien addition chromosome. The normally coloured seed was genetically identical with the Z line and the blue colour served as a marker of those seeds containing the alien addition chromosome, that is, the Y line.

The harvested seed was delivered to a commercial seed sorting machine which was tuned to discriminate between the differently coloured seed and to separate them physically. Using a Sortex 5000 (Trade Mark) seed sorting apparatus, the seed was separated into 35% white and 65% blue seed.

The separated Y line was effectively recycled to the first step of production and the bulk of the product, the Z line, was used in production of fertile F1 hybrids by crossing with any selected normal female parent.

EXAMPLE 2.

TRANSLOCATION INVOLVING CHROMOSOME 4 OF AGROPYRON ELONGATUM AND CHROMOSOME 4B OF BREAD WHEAT

A translocation (4Ag/4B) was crossed into a male sterile Vulcan background. Vulcan is an Australian wheat cultivar, and was converted to male sterility by backcrossing the recessive male sterile gene PROBUS into it.

From this point 2 different procedures were followed:

1. The progeny of blue seed was bulked and sent to be colour sorted. The result of this mechanical sorting (using a SORTEX 5000 sorter) was 65% blue seed and 35% white or non-blue seed. The purity of the non-blue seed was quite satisfactory.

2. In a separate experiment, single plants were identified as heterozygous at the blue locus. These were harvested and blue and white seed were sorted by hand to determine the segregation ratio. In individual heads, the proportion of blue seeds ranged from 45% to 85%. However, the average proportion of blue seed per plant was approximately 65%.

EXAMPLE 3.

TRANSMISSION RATES INVOLVING THE TRANSLOCATION CF24.

The translocation, CF24 was produced by crossing a wheat line carrying chromosome 4 of Agropyron elongatum (blue seed) to a wheat line carrying chromosome 4 of Triticum monococcum (blue seed). Progeny were selected for male fertility and intensity of the blue aleurone. Fertile plants were crossed to male sterile Vulcan and male sterile Skua (Skua is an Australian wheat cultivar, male sterility was produced as described above.)

Segregation ratios in small populations indicated that plants heterozygous at the blue locus would produce approximately 70% blue seed and 30% non-blue seed. In classical Mendelian studies, the dominant character - in this case the blue aleurone - is expected to be expressed in 75% of progeny (hence a 3:1 ratio of dominant to recessive). However, the observed ratio of 7:3 and 13:7 (65:35) in Example 2, simply indicate that the

translocations are not normally transmitted, through the gametes.

EXAMPLE 4.

TRANSMISSION RATES INVOLVING THE TRANSLOCATION CF22.

The translocation CF22, was produced using the same process as described in Example 3. However, while chromosome 4 of Agropyron elongatum is included, male fertility restoration is provided by the short arm of chromosome 4 of Triticum monococcum (in the initial cross only the short arm of chromosome 4 of T. monococcum was present, ie, it was a telocentric addition).

In small populations, the transmission rate of the translocation chromosome CF22 appears to be 70%. A line designated 89-130(I) is a cross between male sterile Skua and CF22. This line has constantly produced very good blue seed and white seed in a 3:1 ratio in small populations.

EXAMPLE 5.

TRANSMISSION RATES INVOLVING THE TRANSLOCATION CF30.

The translocation CF30 was produced in the same manner as CF22 and carries the same components; i.e. blue aleurone is provided by chromosome 4 of Agropyron elongatum and male fertility is provided by the short arm of chromosome 4 of T. monococcum. So the translocation is expected to be 4Ag/4mt, as in CF22.

In small populations, the blue marker was transmitted through 33% of progeny from a self pollinated plant, heterozygous for CF30. This result can be explained by the different components of the translocation. The amount of chromatin present in the translocation from 4Ag and 4mt will effect the transmission of the translocation through both male and female gametes.

EXAMPLE 6.

COMPARISON OF ADDITION AND SUBSTITUTION PLANT LINES

A calculation can be made that compares the seed production in accordance with this invention, using the addition and substitution schemes. The calculation assumes that 1 plant produces 100 seeds, that 4000 seeds weigh 1 kg, and that 35kg/ha of seed produces 1 tonne of wheat grain.

1. Using an addition plant line, and the system shown in Figure 2, if it is assumed that the male transmission rate is 10%, and the female transmission rate is 20%, then after 6 generations of an addition line of hybrid wheat, the result will be about 77 tonne of heterozygous blue seed, and 213 tonne of white seed.

2. In comparison, using a substitution plant line, and the system shown in Figure 3 or 4, for example, if it is assumed that the male transmission rate is 40%, and the female transmission rate is 50%, then after 6 generations of a substitution line of hybrid wheat, the result will be about 3906 tonne of heterozygous blue seed, and 2343 tonne of white seed.

EXAMPLE 7.

PRODUCTION OF A SUBSTITUTION LINE

A substitution line was created by the following procedure. A dark blue 43 chromosome line was created following the procedure described in part (a) of Example 1. These plants were self pollinated for 2 generations. Then they were crossed with male-sterile plants. The fertility of the resulting progeny and the segregation ratios for the blue allerone were checked. Those that had a classic Mendelian ratio were selected, as indicating a substitution line. Most likely, a centric fusion or Robertsonian translocation was responsible.

THE CLAIMS

1. A method for the maintenance of a male sterile parental plant line for use in the production of hybrids, which comprises crossing a female parent with a male parent, said female parent being a homozygous male sterile plant, said male parent being isogenic to the female but having a chromosome bearing a dominant male fertility restorer gene and a colour marker gene which confers a characteristic colouration on the progeny seed; harvesting from that cross a population of progeny seed consisting of a mixture of the two parental lines; and physically separating the progeny seed on the basis of the colour marker.

2. The method of claim 1, whereby the male parent isogenic to the female contains an additional chromosome to its usual number bearing a dominant male fertility restorer gene and a colour marker gene.

3. The method of claim 1, whereby if the male parent contains an additional chromosome to its usual number, it is propagated until a substitution occurs to produce a substitution line having a chromosome bearing a dominant male fertility restorer gene and a colour marker gene which confers a characteristic colouration on the progeny seed; and said substitution line is then used in the production of hybrids.

4. The method of claim 1, whereby the plant line is a wheat or barley plant line.

5. The method of claim 1, whereby the colour marker is a blue aleurone gene.

6. The method of claim 1, whereby the plant line contains chromosome material selected from Triticum aestivum L. and any of Triticum monococcum (4m), Triticum thaoudar (4th) or Triticum urartu (4u), and the colour marker gene is the blue aleurone marker gene from Agropyron elongatum L. (4Ag).

7. A plant line for use in the production of hybrids, having a chromosome bearing a dominant male fertility restorer gene and a colour marker gene which confers a characteristic colouration on the progeny seed, and said progeny seed.

8. The plant line or seed of claim 7, which has at least one additional gene to its usual number.

9. The plant line or seed of claim 7, which has about its usual number of genes.

10. The plant line or seed of claim 7, which is wheat or barley.

11. The plant line of claim 7, whereby the colour marker gene is a blue aleurone gene.

12. The plant line or seed of claim 6, wherein the plant line contains chromosome material selected from Triticum aestivum L. and any of Triticum monococcum (4m), Triticum thaoudar (4th) or Triticum urartu (4u), and the colour marker gene is the blue aleurone marker gene from Agropyron elongatum L. (4Ag).

PRIOR ART

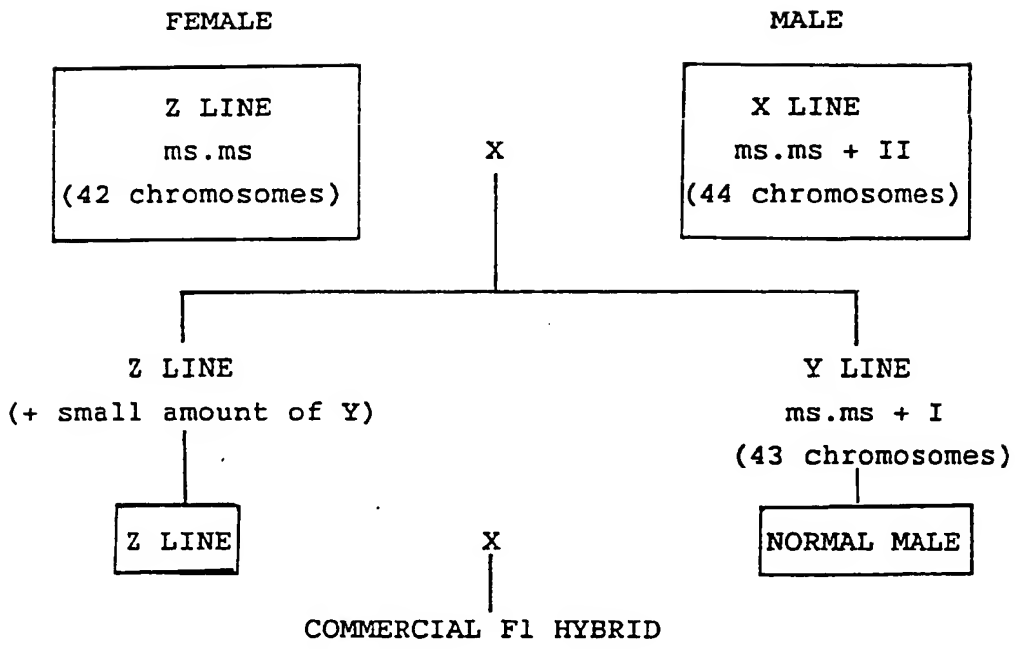



FIGURE 1

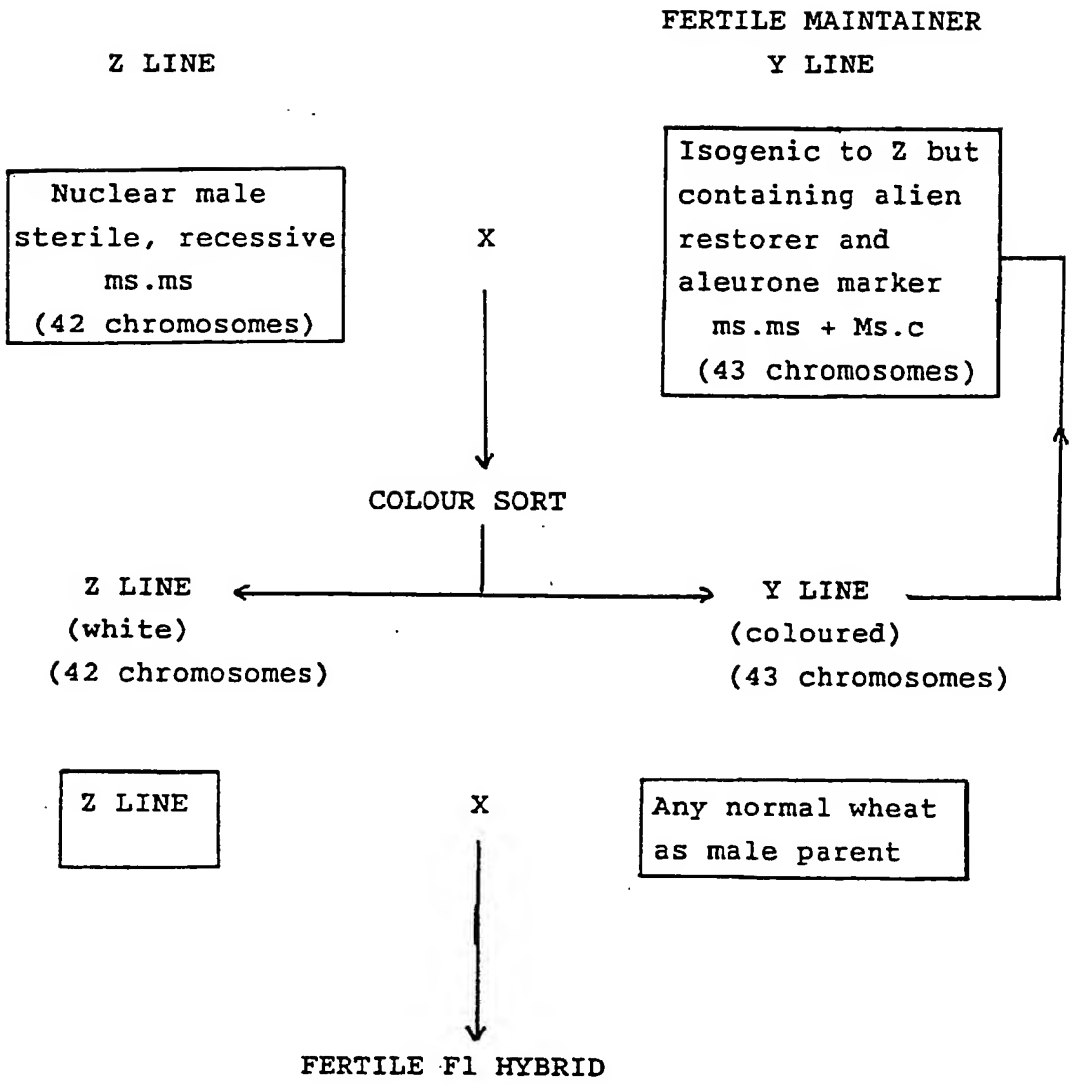
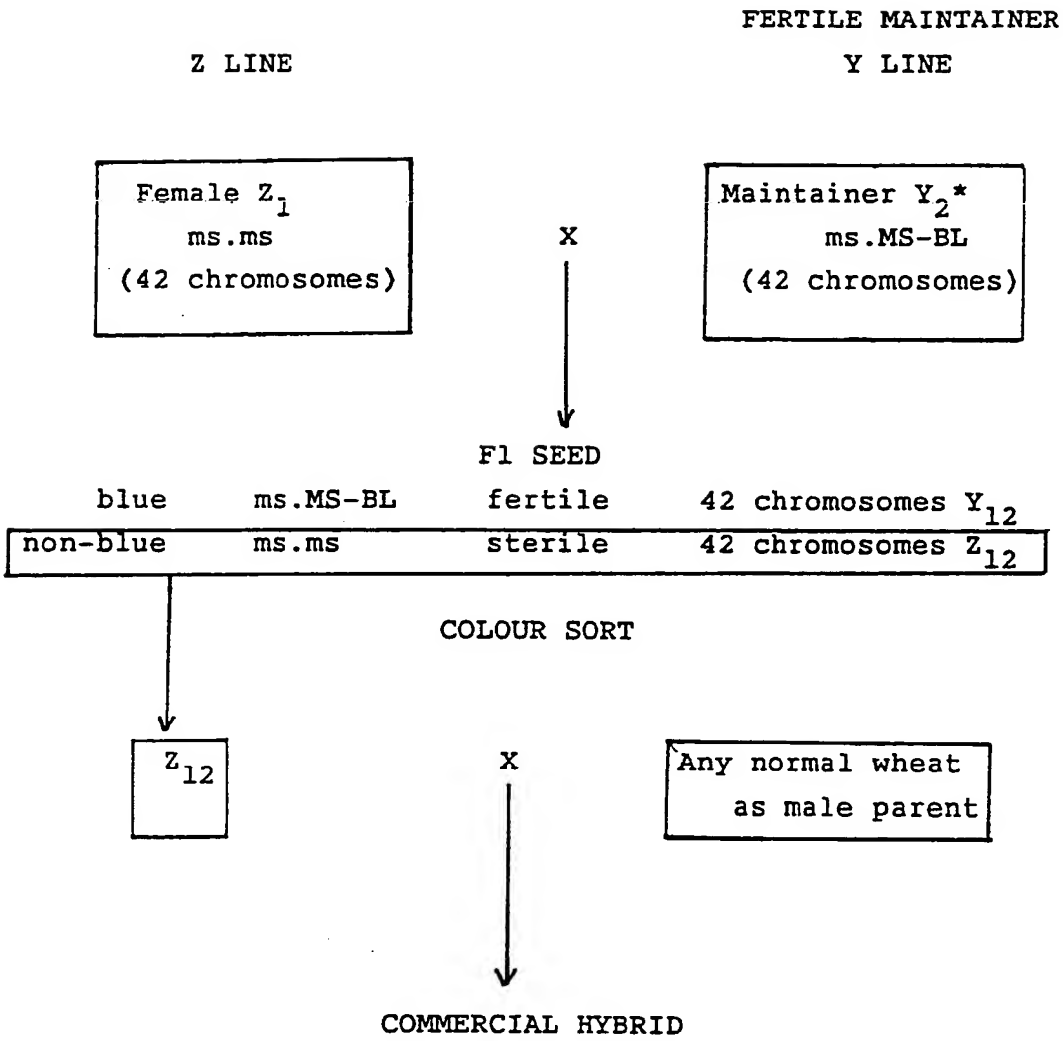

Z LINE
FERTILE MAINTAINER
Y LINE
Nuclear male sterile, recessive ms.ms (42 chromosomes)
X
Isogenic to Z but containing alien restorer and aleurone marker ms.ms + Ms.c (43 chromosomes)
COLOUR SORT
Z LINE (white) (42 chromosomes)
Y LINE (coloured) (43 chromosomes)
Z LINE
X
Any normal wheat as male parent
FERTILE F1 HYBRID

FIGURE 2

3-WAY CROSSES AND COMPLEX CROSS




($Y_2$ is a genetically different line, or parent to $Z_1$, and $Y_1$ is an isoline of $Z_1$)

FIGURE 3

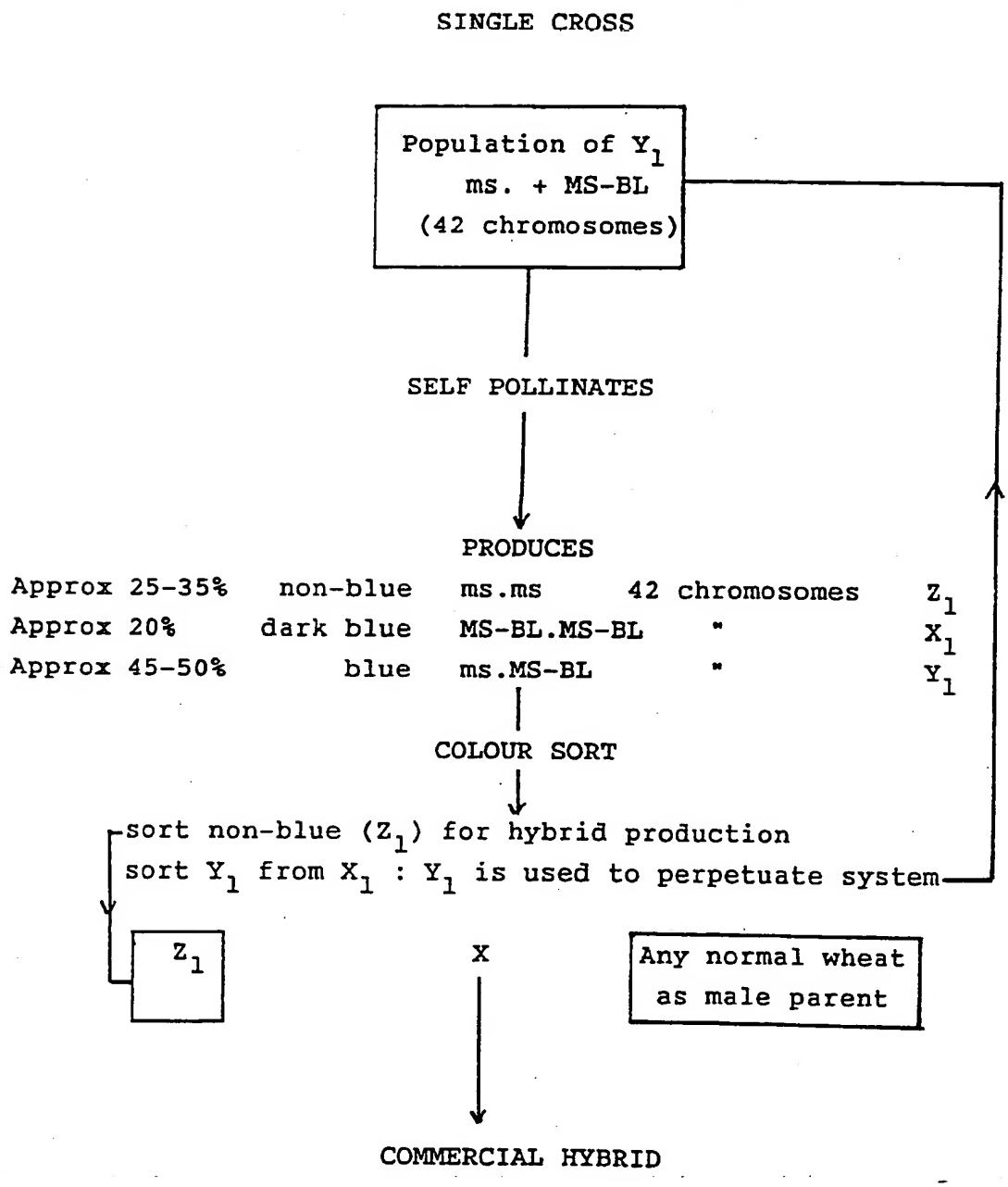

SINGLE CROSS
Population of $Y_1$ ms. + MS-BL (42 chromosomes)
SELF POLLINATES
PRODUCES
Approx 25-35% non-blue ms.ms 42 chromosomes $Z_1$
Approx 20% dark blue MS-BL.MS-BL " $X_1$
Approx 45-50% blue ms.MS-BL " $Y_1$
COLOUR SORT
sort non-blue ($Z_1$) for hybrid production
sort $Y_1$ from $X_1$ : $Y_1$ is used to perpetuate system
$Z_1$
X
Any normal wheat as male parent
COMMERCIAL HYBRID

FIGURE 4

# INTERNATIONAL SEARCH REPORT

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent classification (IPC) or to both National Classification and IPC

Int. Cl.[5] A01H 1/02; 1/04

## II. FIELDS SEARCHED

Minimum Documentation Searched [7]

| Classification System | Classification Symbols |
|---|---|
| WPAT | Keywords: hybrid:, restor:, colour: or color: and A01H |
| CAS 82 | Keywords: as above except A01H |

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

AU:IPC 5 A01H 1/02; 1/04

## III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

| Category* | Citation of Document, [11] with indication, where appropriate of the relevant passages [12] | Relevant to Claim No [13] |
|---|---|---|
| A | US,A,4763441 (DAVIS) 16 August 1988 (16.08.88), Claims, pages 15-22. | 1-12 |
| A | US,A,4727219 (BRAR ET AL.) 23 February 1988 (23.02.88), Claims, pages 12-14. | 1-12 |
| A | AU,B,53246/73 (466787) (GABONATER MESZTESI KUTATO INTEZET) 19 September 1974 (19.09.74), Claims, pages 10-11. | 1-3 |
| A | US,A,3710511 (PATTERSON) 16 January 1973 (16.01.73) Whole document | 1-12 |
| A | US,A,2753663 (JONES) 10 July 1956 (10.07.56) Claims. | 1 |

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## IV. CERTIFICATION

| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report |
|---|---|
| 7 November 1991 (07.11.91) | 11 November 91 |
| **International Searching Authority** | **Signature of Authorized Officer** |
| **AUSTRALIAN PATENT OFFICE** | K AYERS |

# ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL APPLICATION NO. PCT/AU 91/00319

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent Document Cited in Search Report | Patent Family Member | | | |
|---|---|---|---|---|
| US 4763441 | BR 8802607 | CN 1031008 | US 3842538 | |
| AU 53246/73 | DE 2310984 | HU 165923 | IT 984414 | |
| US 4727219 | | | | |
| US 2753663 | | | | |
| US 3710511 | | | | |